Project number: 5429
Funding source: Safefood 2004 (04CR-06)
Date: January, 2010
Project dates: Oct 2005 – Oct 2008

Investigation of the presence of anti-nutritional and toxic compounds in "health foods"

Key external stakeholders:

Manufacturers, wholesalers and retailers of health food products; general public; regulatory agencies: DAFF, FSAI, IMB.

Practical implications for stakeholders:

The objective of this project was to investigate the occurrence of microcystin (MC) and aristolochic acid (AA) toxins in algal and herbal products, respectively.

- Methods were developed and validated to detect AA and microcystin toxins, which can be employed to monitor the safety of health foods.
- Contaminated products were detected and removed from the Irish market.
- A number of health alerts were published worldwide including, Ireland, the UK and Canada.

Main results:

- MC toxins were detected in Klamath Lake blue green algae (BGA) products, which are sold in health foods shops throughout the island at concentrations between <0.5 and 3 mg/kg.
- MC toxins were not detected in spirulina BGA products, which may be used as a substitute for Klamath Lake products.
- AA toxins were detected in some herbal preparations sold on the island but these products have been removed from the market.

Opportunity / Benefit:

- Stakeholders can now access analytical methods for detecting AA and microcystin toxins.
- A novel biosensor assay was developed for detecting microcystin toxins, which has the potential to be exploited as a rapid test.

Collaborating Institutions:

Xenosense Ltd., Belfast.

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1. Project background:

This project was a targeted project call of toxins in health food products from ***safe*food**, the Food Safety Promotion Board. The project was to carry out research on two groups of toxins, namely, microcystin toxins in blue green algae food supplements and aristolochic toxins in herbal products. Prior to this research there was no knowledge of the presence of either toxins in heatlh foods or herbal products in the Irish context. Teagasc and Xenosense Ltd., submitted a project application for this call and were funded to carry out research on this topic. There has been particular concern over the presence of toxins, such as microcystins in food supplements produced from blue-green algae and aristolochic acid in Chinese herbal medicines. In a survey carried out by Health Canada, it was found that microcystin residues were not detected in blue-green algal products containing only the blue-green algae Spirulina. However, for many non-spirulina blue-green algae products, particularly those harvested from natural lakes, when consumed according to manufacturers' directions, the resulting daily intake of microcystins was above the level considered acceptable by Health Canada and the World Health Organisation (WHO). Health Canada is currently determining whether products containing aristolochic acid are being sold on the Canadian market.

Microcystin toxins and blue green algae

Blue-green algae are belong to a type of photosynthetic bacteria, cyanobacteria, that rely on sunlight for energy. Cyanobacteria form in shallow, warm, slow moving or still water. They are made up of cells, which can house poisons called cyanobacterial toxins. Microcystins are the most common of the cyanobacterial toxins found in water and are extremely stable over a range of temperature and pH levels. There have been approximately 65 different microcystin toxins identified in water supplies around the world. Microcystin-LR appears to be the most commonly found microcystin.

The first case of poisoning attributed to blue-green algae was observed at Lake Alexandrina in South Australia in 1878. In that case, livestock died within hours of drinking contaminated water. Human illnesses attributed to cyanobacterial toxins come into three categories: gastroenteritis and related diseases, allergic and irritation reactions, and liver diseases. Microcystins are also tumor-promoting substances. Until recently, there have not been any definite human deaths attributed to cyanobacterial toxins, although there have been a number of circumstantial cases. In the 1920-30s there were more than 1000 cases of poisoning (the so called "Haff-disease") relating to people who had eaten fish from the southern Baltic Sea. Some of those cases resulted in deaths. It is generally believed that microcystin intoxication was involved. The first documented human fatalities caused by microcystin intoxication was in 1996 in a haemodialysis unit in Brazil. Of the 126 patients who developed signs of acute neurotoxicity and subacute hepatoxicity following the use of water from a lake contaminated with a cyanobacteria, 60 patients died. As a result, the World Health Organisation (WHO) has set a provisional guideline value of 1 µg/l microcystin-LR in drinking water.

Aristolochic acids and their toxic effects

Aristolochic acids (AAs) are toxic phytochemicals found in the plant genus Aristolochia within the family aristolochiaceae. Low levels of AAs are found in the species Aristilochia fangchi Wu, which is used in the medicinal agent guang fang ji, a Chinese medicine used to treat painful obstruction due to wind, heat and dampness. Until 1982, AA isolated from A. clematitis was used in some pharmaceutical preparations as an anti-inflammatory agent. Pharmaceutical companies ceased using AA as an anti-inflammatory agent after it was reported that AA was carcinogenic in rats and is also mutagenic in vitro. Since then, several *in vivo* and *in vitro*research data in peer reviewed journals have indicated that AAs have mutagenic and carcinogenic properties. Nephrotoxic and cytotoxic properties of AA had been reported even in the late 1950s and early 1960s. In 1963, Peters and Hedwall observed the loss of concentrating ability in the kidney as a result of intoxication with AA [[1]].

2. Questions addressed by the project:

- Are microcystin toxins present in blue green algae products sold in the Irish market place?

[1] G. Peters and P.R. Hedwall. Arch. Int. Pharmacodyn. (1963) 145 334.

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- Are aristolochic acids of concern the Irish consumer?

3. The experimental studies:

The project team adopted a number of approaches to identify key supply-lines within Ireland. Lists of Health Food Retailers, Herbalists, Traditional Chinese Medicine (TCM) practitioners and other possible suppliers were generated by consulting business directories for the different regions on the island (e.g. goldenpages.ie and yell.com). Various professional trade associations and registers (e.g. Irish Association of Health Stores (IAHS), Irish Health Trade Association (IHTA), Traditional Chinese Medicine Council of Ireland (TCMCI), The Professional Register of Traditional Chinese Medicine (PRTCM)) were consulted to provide background information for the project; primarily to identify the major wholesalers of health food products in Ireland. This was followed up by exploratory visits to pharmacies and health food stores in both Dublin and Belfast. A number of samples were bought for initial method development purposes.

Researchers at Xenosense Ltd., developed and validated a rapid biosensor assay for the detection of microcystin toxins in blue green algae products. This assay was used for the survey part of the project and it was backed up by a LC-MS/MS assay, which was developed and validated at Teagasc, Ashtown. Teagasc researchers developed a sensitive HPLC fluorescence method for the detection of low levels of aristolochic acids (AAs) in herbal products. A confirmatory LC-MS/MS method was also developed to confirm the presence of AAs. A total of 212 BGA food supplements containing Spirulina and A. flos-aquae were purchased from 78 health food shops and 3 pharmacies. All purchased BGAS were screened in the MC10E7 SPR assay for MC presence. Based on the MC10E7 assay data, some samples were selected to be tested in the AD4G2 SPR assay. Determined MC concentrations have been normalized for the MC-LR RM. A total of ten to fifteen tablets or capsules (depending on weight) were taken and ground into a fine powder form or mixed well prior to analysis. The contents of each bottle containing BGAS in powder form was well mixed prior to testing. A total of 366 samples of green teas, specialist teas and chinese herbal products were tested for AA toxins.

4. Main results:

Approximately 200 samples of blue green algae products (Spirulina and Klamath Lake BGAs) were sampled from the health food shops on the island. BGA samples were screened using an immunobiosensor assay that was developed on the project and positive samples were confirmed by LC-MS/MS. The survey of BGA products found that Spirulina samples did not contain microcystin residues above 0.5 mg/kg. The survey of Klamath Lake BGA products found that microcystin toxins could be determined in the majority of samples. LC-MS/MS analysis of products confirmed the presence of Microcystin LR in suspect samples. Microcystin LR residues typically ranged between 0.5 and 4 mg/kg.

400 samples of herbal products were sampled from a range of retail outlets including health food shops, supermarkets and Traditional Chinese Medicine outlets. The herbal products comprised mainly of teas including functional teas and Chinese herbal products. Herbal products were analyzed for aristolochic acid content using a newly developed HPLC fluorescent method and suspect samples were confirmed by LC-MS/MS. The herbal survey found that aristolochic acid residues (AAI and AAII) were not present in tea samples (LOQ of method: 10 and 22.4 µg/kg for AAI and 22.4 AAII, respectively). Analysis of Chinese herbal products found the presence of AA residues in four herbal preparations (Xi Gan Wan, Chuan Xiong Cha Tiao Wan, Bai Tou Weng Wan and Xiao Qin Long Wan). More recently, analysis of raw herbs found that AAI and AAII was present in samples of Asarum plant, which is commonly known as Xi Xin.

5. Opportunity/Benefit:

The nature of the technology developed on this project is such that it can be applied in any laboratory that has equivalent equipment and therefore is not suitable for patenting or licensing. A workshop was held to disseminate the finding of the research at AFRC to stakeholders. Researchers at AFRC have liaised with regulatory agencies concerning the research findings. A number of contaminated herbal products have been removed from the market as a result of the research. The technology developed on the project can be used as a tool for any further investigation of the marketplace.

6. Dissemination:

Main publications:

Vinogradova, T, Danaher, M., Baxter, A, Moloney, M., Victory, D., Haughey, S.A. Rapid surface plasmon

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resonance immunobiosensor assay for microcystin toxins in blue-green algae food supplements. Talanta 84 (2011) 638.

Popular publications:

- Danaher, M and Victory, D. (2007) Sensitive method for the determination of aristolochic acids in teas using HPLC with fluorescence detection. In proceedings of: 121st AOAC International Annual Meeting and Exposition, California, Anaheim, USA, 16-20th September 2007.
- Vinogradova, T., Haughey, S.A., Baxter, G.A. Victory, D. and Danaher. M. Development and Validation of an SPR Biosensor Assay for Microcystins in Blue-Green Algae Food Supplements. In proceedings of: 121st AOAC International Annual Meeting and Exposition, California, Anaheim, USA, 16-20th September 2007.
- Danaher, M and Victory, D (2007) Automated extraction of Microcystin-LR residues from blue green algae health food samples with detection by LC-MS/MS. In proceedings of: 3rd International Symposium on Recent Advances in Food Analysis. Prague, Czech Republic, 7-9th November 2007.

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